DATA EVALUATION RECORD

STUDY 1

CHEM 055501 Ethoxyquin §161-1
CAS No. 000091-53-2
FORMULATION--00--ACTIVE INGREDIENT

STUDY ID 43753101
Reynolds, J. and M. Campbell. 1995. Hydrolysis of ^{14}C-ethoxyquin. Laboratory Project ID: XBL 95011. Unpublished study performed by XenoBiotic Laboratories, Inc., Plainsboro, NJ; and submitted by the Oregon, Washington, California Pear Bureau, Liberty, MO.

DIRECT REVIEW TIME = 35 Hours

REVIEWED BY:	M. T. Holdsworth, M.S.	Signature:
TITLE:	Scientist	Date:
EDITED BY:	W.R. Sutton, Ph.D.	Signature:
TITLE:	Senior Scientist	Date:
APPROVED BY:	P. H. Howard	Signature:
TITLE:	Project Manager	Date:
ORG:	Syracuse Research Corp. Arlington, VA 22202	
TEL:	703/413-9369	

APPROVED BY:	Mah Shamin
TITLE:	Branch Chief
ORG:	ERB IV/EFED/OPP
TEL:	703/305-5776

SIGNATURE:

CONCLUSIONS

Degradation - Hydrolysis

1. This study is scientifically valid and provides useful information on the hydrolysis of ethoxyquin. However, material balances showed a general decrease with time for all three pH systems.

2. This study does not meet Subdivision N Guidelines for the fulfillment of EPA data requirements on hydrolysis for the following reasons:

   (i) degradates comprising ≥10% of the applied were isolated but were not definitively identified.

3. Uniformly phenyl ring-labeled [^{14}C]ethoxyquin, at a nominal concentration of 10 ppm, was hydrolytically unstable in pH 5, 7, and 9 sterile, aqueous buffer solutions incubated in darkness at 25 ± 1 °C for 9, 15, and 21 days, respectively. The parent compound degraded with registrant-calculated half-lives of 3.7, 6.7, and 9.3 days for the pH 5, 7, and 9 aqueous buffer solutions, respectively. Several degradation products were formed, labeled as Deg 1-7. The isolated degradates were not definitively identified; tentative identification was in the form of proposed structures. In the pH 5 buffer system, the parent compound was initially 100.0% of the applied radioactivity, decreased to 77.2% by 1 day posttreatment, was 53.1% at 3 days, and was 18.2% at 9 days. In the pH 7 buffer system, the parent compound was initially 100.7% of the applied radioactivity, decreased to 76.5% of the applied by 3 days posttreatment, was 51.0% at 7 days, and was at 20.8% at 15 days. In the pH 9 buffer system, the parent compound was initially 100.5% of the applied radioactivity, decreased to 76.4% by 5 days posttreatment, was 54.6% at 10 days, and was 21.2% at 21 days. In the pH 5 buffer system, the major degradate designated DEG-3 (two proposed structures) was initially 3.6% of the applied at 1 day posttreatment, increased to 25.0% by 5 days, and was a maximum of 31.2% at 9 days. The major degradate designated DEG-2 (two proposed structures) was initially 8.8% of the applied radioactivity at 1 day posttreatment, increased to 14.5% by 5 days, and was a maximum of 19.6% at 9 days. The major degradate designated DEG-4 (one proposed structure) was initially 3.3% of the applied radioactivity at 1 day posttreatment, increased to 7.2% by 3 days, was a maximum of 11.2% at 7 days, and was 10.7% at 9 days. All minor degradates were present at ≤4.1% of the applied radioactivity throughout the incubation period. In the pH 7 buffer system, the major degradate designated DEG-3 (two proposed structures) was initially 6.5% of the applied radioactivity at 3 days posttreatment, increased to 13.5% by 7 days, and was 26.8% at 15 days. The major degradate designated DEG-2 (two proposed structures) was initially 8.1% of the applied radioactivity at 3 days posttreatment, increased to 11.4% by 7 days, and was a maximum of 15.1 % at 15 days. The major degradate designated DEG-1 (two proposed structures) was initially 3.2% of the applied radioactivity at 5 days posttreatment, increased to 7.8%

by 9 days, and was a maximum of 13.3% at 15 days. The minor degradate designated DEG-4 (one proposed structure) was a maximum of 6.9% of the applied radioactivity at 15 days posttreatment. All other minor degradates were present at ≤3.6% of the applied radioactivity throughout the incubation period. In the pH 9 buffer system, the major degradate designated DEG-3 (two proposed structures) was initially 6.3% of the applied radioactivity at 5 days posttreatment, increased to 12.9% by 10 days, and was a maximum of 23.9% at 21 days. The major degradate designated DEG-1 (two proposed structures) was initially 3.3% of the applied radioactivity at 5 days posttreatment, increased to 9.8% by 10 days, and was a maximum of 18.4% at 21 days. The major degradate designated DEG-2 (two proposed structures) was initially 6.4% of the applied radioactivity at 5 days posttreatment, increased to 10.3% by 10 days, and was 15.3% at 21 days. The minor degradate designated DEG-4 (one proposed structure) was a maximum of 4.1% of the applied radioactivity at 21 days posttreatment. All other minor degradates were present at ≤3.6% of the applied radioactivity.

METHODOLOGY

Uniformly phenyl ring-labeled [^{14}C]ethoxyquin {(quinoline, 6-ethoxy-1,2-dihydro-2,2,4-trimethyl-); radiochemical purity 95.7%, specific activity 4.11 mCi/mmol; Table 1, p. 26}, dissolved in acetonitrile/methanol, was added at a nominal concentration of 10 ppm to pH 5 (acetate), pH 7 [tris (hydroxymethyl) aminomethane], and pH 9 (boric acid) 0.01 M aqueous buffer solutions (pp. 12, 13). The buffered solutions were added to individually labeled and sterilized vials. The vials were capped and incubated in a constant temperature incubator at 25 ± 1 °C in darkness (Appendix C, p. 91). The pH was monitored throughout the incubation period in control vials; data were not reported. Microbial evaluation of control vials was conducted to determine sterility in the test samples (Appendix K; pp. 155-160). Other control vials were purged with nitrogen and were analyzed at the study termination for each pH system. Duplicate samples were removed for analysis at 0, 1, 2, 3, 5, 7 and 9 days for the pH 5 buffer systems; 0, 3, 5, 7, 9, 12 and 15 days for the pH 7 buffer systems; and 0, 5, 7, 10, 14 and 21 days for the pH 9 buffer systems.

At each sampling interval, duplicate aliquots of the test solutions were analyzed for total radioactivity by LSC. Each sample was extracted twice by partitioning with CH_2Cl_2 (p. 15). The extract solution was concentrated using a nitrogen stream and partially reconstituted with CH_3OH:CH_2Cl_2 (1:1, v:v) for chromatographic analysis. Test solutions were analyzed directly by HPLC (Waters μBondpack C18 column) with a mobile phase gradient of 0.4% formic acid:CH_3OH (95:5, 35:65, 0:100; v:v; p. 16). Test samples were co-chromatographed with nonradiolabeled reference standards which were visualized under UV (254 nm) light. To confirm compound identities, LC/MS (Waters Nova-Pak C18 column) analysis with a mobile phase gradient of 0.4% formic acid:CH_3OH (95:5, 10:90, 0:100; v:v) or (Waters μBondpack C18 column) with a mobile phase gradient of

0.4% formic acid:CH_3OH (95:5, 35:65, 0:100; v:v) and GC/MS (RTX-1 column) analysis in EI and CI mode were performed.

DATA SUMMARY

Uniformly phenyl ring-labeled [^{14}C]ethoxyquin (radiochemical purity 95.7%), at a nominal concentration of 10 ppm, was hydrolytically unstable in pH 5, 7, and 9 sterile, aqueous buffer solutions incubated in darkness at 25 ± 1 °C for up to 9, 15, and 21 days, respectively. The parent compound degraded with registrant-calculated half-lives of 3.7, 6.7, and 9.3 days in the respective pH 5, 7, and 9 aqueous buffer solutions (Figures 31-33, pp. 71-73). Several degradation products were formed, labeled as Deg 1-7. The isolated degradates were not definitively identified; tentative identification was in the form of proposed structures. In the pH 5 buffer system, the parent compound was initially present at 100.0% of the applied radioactivity, decreased to 77.2% of the applied by 1 day posttreatment, was present at 53.1% of the applied at 3 days, was 25.6% of the applied at 7 days, and was present at 18.2% of the applied at 9 days (Table VIII, p. 33). In the pH 7 buffer system, the parent compound was initially present at 100.7% of the applied radioactivity, decreased to 76.5% of the applied by 3 days posttreatment, was present at 51.0% of the applied at 7 days, was 31.1% of the applied at 12 days, and was present at 20.8% of the applied at 15 days (Table X, p. 35). In the pH 9 buffer system, the parent compound was initially present at 100.5% of the applied radioactivity, decreased to 76.4% of the applied by 5 days posttreatment, was present at 54.6% of the applied at 10 days, was 39.5% of the applied at 14 days, and was present at 21.2% of the applied at 21 days (Table XII, p. 37). In the pH 5 buffer system, the major degradate designated

DEG-3 (two proposed structures)

was initially present at 3.6% of the applied at 1 day posttreatment, increased to 25.0% of the applied by 5 days, and was present at a maximum of 31.2% of the applied at 9 days. The major degradate designated

DEG-2 (two proposed structures)

was initially present at 8.8% of the applied radioactivity at 1 day posttreatment, increased to 14.5% of the applied by 5 days, and was present at a maximum of 19.6% of the applied at 9 days. The major degradate designated

DEG-4 (one proposed structure)

was initially present at 3.3% of the applied radioactivity at 1 day posttreatment, increased to 7.2% of the applied by 3 days, was present at a maximum of 11.2% of the applied at 7 days, and was present at 10.7% of the applied at 9 days. All other minor degradates were

present at ≤4.1% of the applied radioactivity. In the pH 7 buffer system, the major degradate designated

DEG-3 (two proposed structures)

was initially present at 6.5% of the applied radioactivity at 3 days posttreatment, increased to 13.5% of the applied by 7 days, and was present at a maximum of 26.8% of the applied at 15 days. The major degradate designated

DEG-2 (two proposed structures)

was initially present at 8.1% of the applied radioactivity at 3 days posttreatment, increased to 11.4% of the applied by 7 days, and was present at a maximum of 15.1 % of the applied at 15 days. The major degradate designated

DEG-1 (two proposed structures)

was initially present at 3.2% of the applied radioactivity at 5 days posttreatment, increased to 7.8% of the applied by 9 days, and was present at 13.3% of the applied at 15 days. The minor degradate DEG-4 (one proposed structure) was present at a maximum of 6.9% of the applied radioactivity at 15 days posttreatment. All other minor degradates were present at ≤3.6% of the applied radioactivity throughout the incubation period. In the pH 9 buffer system, the major degradate designated

DEG-3 (two proposed structures)

was initially present at 6.3% of the applied radioactivity at 5 days posttreatment, increased to 12.9% of the applied by 10 days, and was present at a maximum of 23.9% of the applied at 21 days. The major degradate designated

DEG-1 (two proposed structures)

was initially present at 3.3% of the applied radioactivity at 5 days posttreatment, increased to 9.8% of the applied by 10 days, and was present at a maximum of 18.4% of the applied at 21 days. The major degradate designated

DEG-2 (two proposed structures)

was initially present at 6.4% of the applied radioactivity at 5 days posttreatment, increased to 10.3% of the applied by 10 days, and was present at a maximum of 15.3% of the applied at 21 days. The minor degradate DEG-4 (one proposed structure) was present at a maximum of 4.1% of the applied radioactivity at 21 days posttreatment. All other minor degradates were present at ≤3.6% of the applied radioactivity.

The material balances (for individual replicates) for the pH 5, 7, and 9 test systems were 88.9-101.8%, 85.7-100.8%, and 88.0-102.2%, respectively (Tables VII, IX, and XI; pp. 32, 34, 36). Material balances showed a general decrease with time for all three pH systems.

COMMENTS

1. The degradates in all buffer solutions were not identified, but proposed structures were provided as tentative identification (pp. 21-23). Under Subdivision N Guidelines, the registrant must demonstrate that a reasonable attempt was made to identify all degradates present at ≥10% of the application.

2. Material balances, based on the sum of parent plus all degradates detected (rather than on total radioactivity present), showed a general decrease with time for all pH systems.

3. The pH was monitored for all buffered test solutions; however, but the data were not reported and the reviewer could not confirm that the pH of the respective systems was held constant (p. 15).

4. Method detection limits were not reported for HPLC or GC/MS analysis. Method detection limits and limits of quantitation should be reported to allow the reviewer to evaluate the adequacy of the methods for determination of parent and metabolite in the test system.

5. The solubility of the test compound for each of the three buffer systems was not reported. The reviewer noted that the test compound was dissolved in acetonitrile/methanol at less than 1% by volume (p. 14). The solubility of the test compound in the three buffer systems should be reported to allow the reviewer to accurately assess whether the compound was available in solution for hydrolytic degradation.

INTRODUCTION

The objective of this study was to assess the hydrolytic stability of Ethoxyquin in aqueous solution buffered at pH 5, 7, and 9 under sterile conditions. (Refer to the *Study Protocol*, Appendix A.) The study was conducted in accordance with the Pesticide Assessment Guidelines of the U.S. Environmental Protection Agency (EPA), Subdivision N, § 161-1.

MATERIALS

A. Test Material and Reference Chemicals

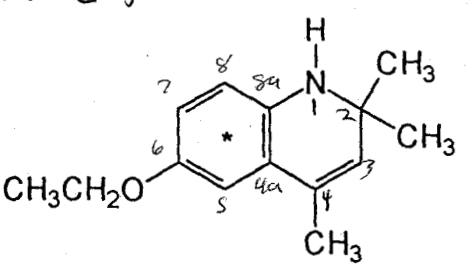
Ethoxyquin labeled with carbon-14 in the ring was used in this study. The origin, specific activity, and radiochemical purity of the ^{14}C-labeled Ethoxyquin used are listed in Table I. (Refer to Appendix B for a representative radiopurity chromatogram.)

Quinoline, 6-ethoxy-1,2-dihydro-2,2,4-trimethyl /

uniformly ring-label ?

position of ^{14}C on ring

[U-^{14}C]



* Denotes position of ^{14}C label

^{13}C parent compound (Lot No. CSL-92-363-16-07, purity >98.5%) was used as a reference chemical in high-performance liquid chromatography (HPLC) analyses.

B. Water and Buffered Solutions

1. Water

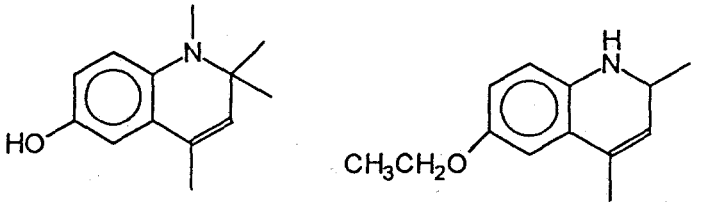
   All water used throughout the experiment was processed through a NANOPure® II (Barnstead Co.) water purification system.

2. Buffered Solutions

   Buffered solutions at pH 5, 7, and 9 were prepared as follows:

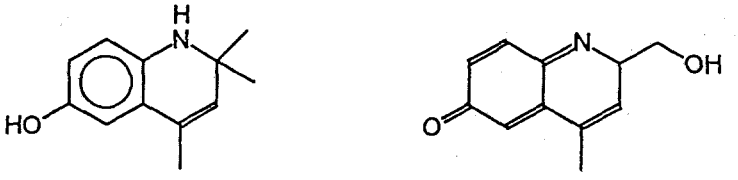
   pH 5: 0.01 *M* sodium acetate (NaOAc) in water adjusted to pH 5 with acetic acid (HOAc)

Deg-1, with a retention time of approximately 12.52 minutes (13 minutes in Tables VIII, X, and XII), appeared in all three pH levels. The percentage of this compound was the lowest in the pH 5 sample, reaching 3.45% by the final harvest. At the other two pH levels, the percentage of radioactivity present in this peak increased steadily throughout the study, reaching a maximum of 13.26% and 18.35% in the pH 7 and pH 9 buffers, respectively. Mass spectral data suggests that Deg-1 has a molecular weight of 203 as indicated in the LC/MS spectra (Figures 14, 15). The first peak detected at R_t = 10.20 min from the LC/MS represents the protonated molecular ion at m/z= 204 (MH^+). There are two possible structures for this degradate as shown below:



Deg-2, with a retention time of approximately 14.10 minutes (14.1 minutes in Tables VIII, X, and XII), appeared in the first sampling interval (other than day 0) and accounted for 8.78%, 8.05%, and 6.42% of the applied dose at pH 5, pH 7, and pH 9, respectively. At the final harvest, the levels of Deg-2 reached a maximum of 19.61% (pH 5), 15.07% (pH 7), and 15.27% (pH 9) of the total applied radioactivity.

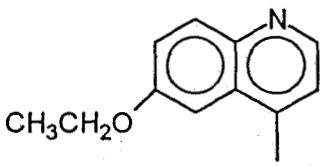
LC/RAM/MS shows a R_t at approximately 10.40 min correlates with that of the Deg-2. Mass spectral data suggest that Deg-2 has a molecular weight of 189 as indicated in the LC/MS spectra (Figures 14, 16). The peak detected at R_t = 10.40 min from the LC/MS represents the protonated molecular ion at *m/z*= 190 (MH^+). Two possible structures for this degradates could be proposed as follows:

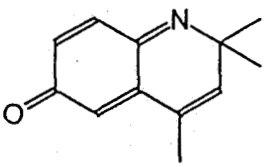


Deg-3, the major degradate, had a retention time of approximately 16.27 minutes (16.3 minutes in Tables VIII, X, and XII). Deg-3 also appeared very early in all samples and reached a maximum of 31.17% (pH 5), 26.83% (pH 7), and 23.88% (pH 9) of the applied radioactivity in the final interval.

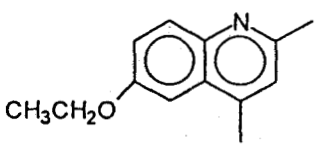
LC/RAM/MS shows a R_t at 13.17 correlates with the R_t of the Deg-3. Mass spectral data suggest that Deg-3 has a molecular weight of 187 as indicated in the LC/MS spectra (Figures 14, 17). The peak detected at R_t = 13.17 min from the LC/MS represents the protonated molecular ion of Deg-3 at *m/z* = 188 (MH^+).

There are also two possible structures for this degradates, shown as follows:



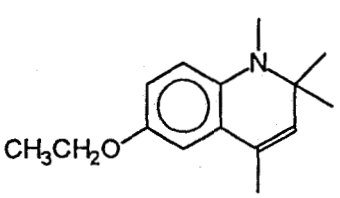


LC/RAM/MS shows parent compound (i.e., Ethoxyquin) at a R_t ~12 min (Figure 13), which corresponds to LC/UV/RAM R_t of 15.45 min (Figure 12). The molecular ion of 217 was confirmed by mass ion at *m/z* 218 (MH^+). Under this peak, there was a minor co-eluting component observed with a mass ion *m/z* = 202 (Figures 14, 18). The protonated mass ion at 202 indicates the molecular ion should be 201. The proposed structure for this degradate is shown below:



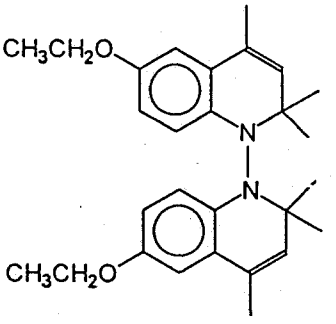
Low levels of Deg-4 (retention time of approximately 17 minutes) initially were detected at the first harvest interval after zero time for all three pH buffers. These levels steadily increased in each of the buffers, reaching a maximum of 10.66% of dosed ^{14}C in the pH 5 buffer. The maximum amount detected in the pH 7 buffer was 6.85%, although less than 5% was observed in the pH 9 buffered solution.

LC/RAM/MS shows a R_t at 15.25 min correlates with the R_t of Deg-4. Mass spectral data suggest that Deg-4 has a molecular weight of 231 as indicated in the LC/MS spectra (Figures 14, 19). The peak detected at R_t = 15.25 min from the LC/MS represents the protonated molecular ion at *m/z*= 232 (MH^+). This product is 14 mass units higher than that of the parent Ethoxyquin (217), indicating the addition of a methyl (CH_3) group, which most likely is on the nitrogen atom as shown below:



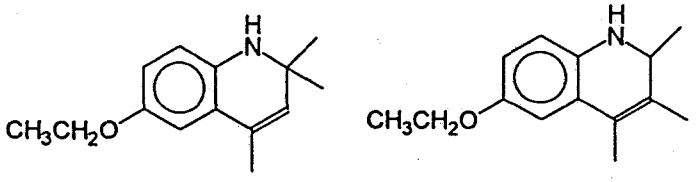
The degradate, designated as Deg-7, with a retention time of approximately 29.23 minutes (30 minutes in Tables VIII, X, and XII) in Figure 12, was observed in all samples harvested except zero time. LC/RAM/MS shows a R_t at 25.44 min that correlates with the R_t of Deg-7. Mass spectral data suggest that Deg-7 has a

molecular weight of 432 as indicated in the LC/MS spectra (Figures 14, 20). The peak detected at R_t = 25.44 min from the LC/MS represents the protonated molecular ion at *m/z*= 433 (MH^+). This product is a dimeric Ethoxyquin (217 x 2 -2 = 432), most likely a N-N bond dimeric product. The proposed structure of Deg-7 is shown as follows:



GC/MS analyses of the CH_2Cl_2 extracts by both EI and CI mode confirmed the presence of most of the previously mentioned degradates. Figures 21–30 show both EI and CI mass spectra for each of the degradates as well as the parent compound.

Deg-1 and Deg-4 were not observed in GC profile (pH 9 sample), suggesting that the degradates might be thermally labile. In addition, the appearance of more than one component having the same molecular weight of 217, which is the same as that of parent compound, indicates the possibility of intramolecular thermal rearrangement as shown below. This observation is consistent with that observed in a previously conducted pear metabolism study (Reference 1).



F. Analysis of Nitrogen Purge Samples

Analysis of the nitrogen-purged samples, which were harvested at the final interval for each pH level, showed a very slight retardation in the hydrolytic degradation of Ethoxyquin. This demonstrates that air oxidation causes a negligible portion of the degradation. There was no significant difference between the degradates observed in the nitrogen-purged samples and those in the samples that were not purged.

G. Validation of RP-HPLC Recovery

Validation of HPLC recovery was conducted by collecting the HPLC eluate in 0.5 minute intervals and counting the samples in the scintillation counter. The data

Table I

Summary of Test Substance and Reference Chemicals

[Ring-U-^{14}C] Ethoxyquin	
Label Position	Ring-U-^{14}C
Reference Number	X336:3A
Lot Number	CSL 94-519-61-28
Specific activity (mCi/mmol)	4.11
Purity	95.66%[1]
Use in Study	Dose samples for preliminary and definitive studies (10 ppm).
[Ring-U-^{13}C] Ethoxyquin	
Lot Number	CSL 92-363-16-07
Purity	>98.5%
Use in Study	HPLC reference chemical

[1]Purity as checked by XenoBiotic Laboratories, Inc. (HPLC 95011007.D01-.D03) and liquid scintillation counting (LSC336:22,24).

Table VI

Summary of Sample Recovery from pH 5, pH 7, and pH 9 Buffers

Interval	pH 5 Sodium Acetate Buffer
0	100.91
1	98.39
2	97.62
3	99.32
5	97.91
7	95.95
9	96.83
Nitrogen Purge	97.22

Interval	pH 7 Tris(hydroxymethyl) Buffer
0	101.34
3	101.50
5	101.86
7	100.83
9	100.33
12	102.15
15	101.13
Nitrogen Purge	101.52

Interval	pH 9 Boric Acid Buffer
0	101.68
5	98.83
7	98.13
10	98.29
14	98.21
21	97.26
Nitrogen Purge	98.79

Note: Data summarized from Table III - V.

Table VIII

Average Percent Distribution of Parent Compound and Degradates in pH 5 Buffer

CH_2Cl_2-1	Ethoxyquin	Deg-1 (13)	Deg-2 (14.1)	Deg-3 (16.3)	Deg-4 (17)	Deg-5 (18.5)	Deg-6 (23)	Deg-7 (30)	Total
Day	% of Applied	% of Applied	% of Applied	% of Applied	% of Applied	% of Applied	% of Applied	% of Applied	% of Applied
0	100.02	ND	ND	ND	ND	ND	ND	ND	100.02
1	77.24	0.76	8.78	3.56	3.27	ND	ND	2.31	95.91
2	66.06	ND	11.54	8.77	5.76	ND	ND	1.89	94.02
3	53.10	1.03	15.23	15.93	7.19	ND	ND	2.36	94.82
5	36.57	1.65	14.49	25.02	10.71	ND	ND	2.52	90.95
7	25.59	2.90	15.05	29.34	11.19	2.30	ND	2.87	89.21
9	18.18	3.45	19.61	31.17	10.66	4.08	ND	2.47	89.60
9 N_2	23.94	3.05	18.13	29.18	11.73	1.31	ND	3.26	90.59

ND = Not Detected

Note:Refer to Table VII for Replicate A and Replicate B data.

Table X

Average Percent Distribution of Parent Compound and Degradates in pH 7 Buffer

CH_2Cl_2-1	Ethoxyquin	Deg-1 (13)	Deg-2 (14.1)	Deg-3 (16.3)	Deg-4 (17)	Deg-5 (18.5)	Deg-6 (23)	Deg-7 (30)	Total
Day	% of Applied	% of Applied	% of Applied	% of Applied	% of Applied	% of Applied	% of Applied	% of Applied	% of Applied
0	100.70	ND	ND	ND	ND	ND	ND	ND	100.70
3	76.49	ND	8.05	6.47	3.03	0.67	ND	2.52	97.22
5	62.66	3.24	10.93	9.73	3.47	ND	1.23	3.46	94.70
7	51.03	4.39	11.37	13.47	5.02	1.21	1.30	2.62	90.39
9	41.53	7.81	11.55	18.74	4.41	1.22	ND	3.58	88.83
12	31.12	12.48	14.04	20.71	6.11	ND	ND	3.22	87.67
15	20.76	13.26	15.07	26.83	6.85	ND	ND	3.01	85.77
15 N_2	26.10	12.45	12.41	26.09	6.92	ND	ND	2.89	86.86

ND = Not Detected

Note:Refer to Table IX for Replicate A and Replicate B data.

Table XII

Average Percent Distribution of Parent Compound and Degradates in pH 9 Buffer

CH_2Cl_2-1	Ethoxyquin	Deg-1 (13)	Deg-2 (14.1)	Deg-3 (16.3)	Deg-4 (17)	Deg-5 (18.5)	Deg-6 (23)	Deg-7 (30)	Total
Day	% of Applied	% of Applied	% of Applied	% of Applied	% of Applied	% of Applied	% of Applied	% of Applied	% of Applied
0	100.47	ND	ND	ND	ND	ND	ND	ND	100.47
5	76.39	3.32	6.42	6.34	1.32	ND	ND	1.97	95.74
7	66.75	5.49	8.71	9.02	2.15	ND	ND	1.84	93.94
10	54.59	9.79	10.27	12.94	3.52	ND	ND	2.00	93.10
14	39.54	14.44	11.29	19.23	3.69	ND	ND	3.08	91.25
21	21.24	18.35	15.27	23.88	4.10	1.80	ND	3.52	88.16
21 N_2	28.80	17.63	15.43	21.55	4.19	ND	ND	2.86	90.46

ND = Not Detected

Note:Refer to Table XI for Replicate A and Replicate B data.

Table XIII

Percent Remaining and Half-life Regression Analysis for ^{14}C-Ethoxyquin in pH 5 Buffered Solution

Days	^{14}C-Ethoxyquin ln(% Rem.)[1]	^{14}C-Ethoxyquin % Remaining
0	4.60537	100.02
1	4.34692	77.24
2	4.19056	66.06
3	3.97218	53.10
5	3.59923	36.57
7	3.24220	25.59
9	2.90032	18.18

Regression: 0-9 Days	
Equation: ln(^{14}C-Ethoxyquin) = C - k * Time	
Determination Coeff:	0.99793
Coeff. of Correlation:	0.99896
Std. Error of Est:	0.03075
Constant (C):	4.55934
Rate Constant (k):	0.18736
Half-life = ln(2) / k =0.693 / k =	3.70 (Days)

[1]The ln values were not rounded, 15 digit precision was used.

Note:14C-Ethoxyquin % Remaining was obtained from Table VIII.

Table XIV

Percent Remaining and Half-life Regression Analysis for ^{14}C-Ethoxyquin in pH 7 Buffered Solution

Days	^{14}C-Ethoxyquin ln(% Rem.)[1]	^{14}C-Ethoxyquin % Remaining
0	4.61215	100.70
3	4.33716	76.49
5	4.13772	62.66
7	3.93241	51.03
9	3.72642	41.53
12	3.43785	31.12
15	3.03303	20.76

Regression: 0-15 Days	
Equation: ln(^{14}C-Ethoxyquin) = C - k * Time	
Determination Coeff:	0.99648
Coeff. of Correlation:	0.99824
Std. Error of Est:	0.03511
Constant (C):	4.64539
Rate Constant (k):	0.10394
Half-life = ln(2) / k =0.693 / k =	6.67 (Days)

[1]The ln values were not rounded, 15 digit precision was used.

Note:14C-Ethoxyquin % Remaining was obtained from Table X.

Table XV

Percent Remaining and Half-life Regression Analysis for ^{14}C-Ethoxyquin in pH 9 Buffered Solution

Days	^{14}C-Ethoxyquin ln(% Rem.)[1]	^{14}C-Ethoxyquin % Remaining
0	4.60986	100.47
5	4.33585	76.39
7	4.20095	66.75
10	3.99985	54.59
14	3.67731	39.54
21	3.05589	21.24

Regression: 0-21 Days	
Equation: ln(^{14}C-Ethoxyquin) = C - k * Time	
Determination Coeff:	0.98910
Coeff. of Correlation:	0.99454
Std. Error of Est:	0.06432
Constant (C):	4.68908
Rate Constant (k):	0.07465
Half-life = ln(2) / k =0.693 / k =	9.28 (Days)

[1]The ln values were not rounded, 15 digit precision was used.

Note:14C-Ethoxyquin % Remaining was obtained from Table XII.

Figure 1

Extraction Scheme

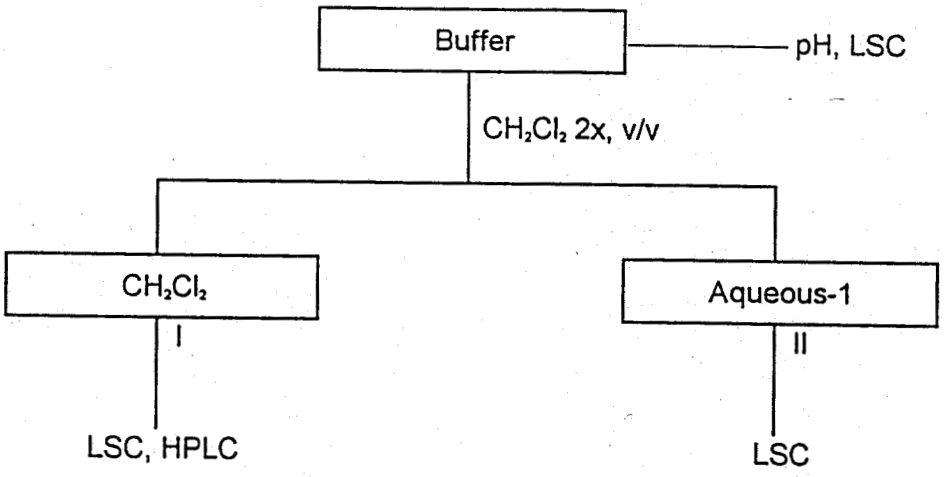


Figure 31

Schematic Illustration of Half-life Regression Analysis for pH 5

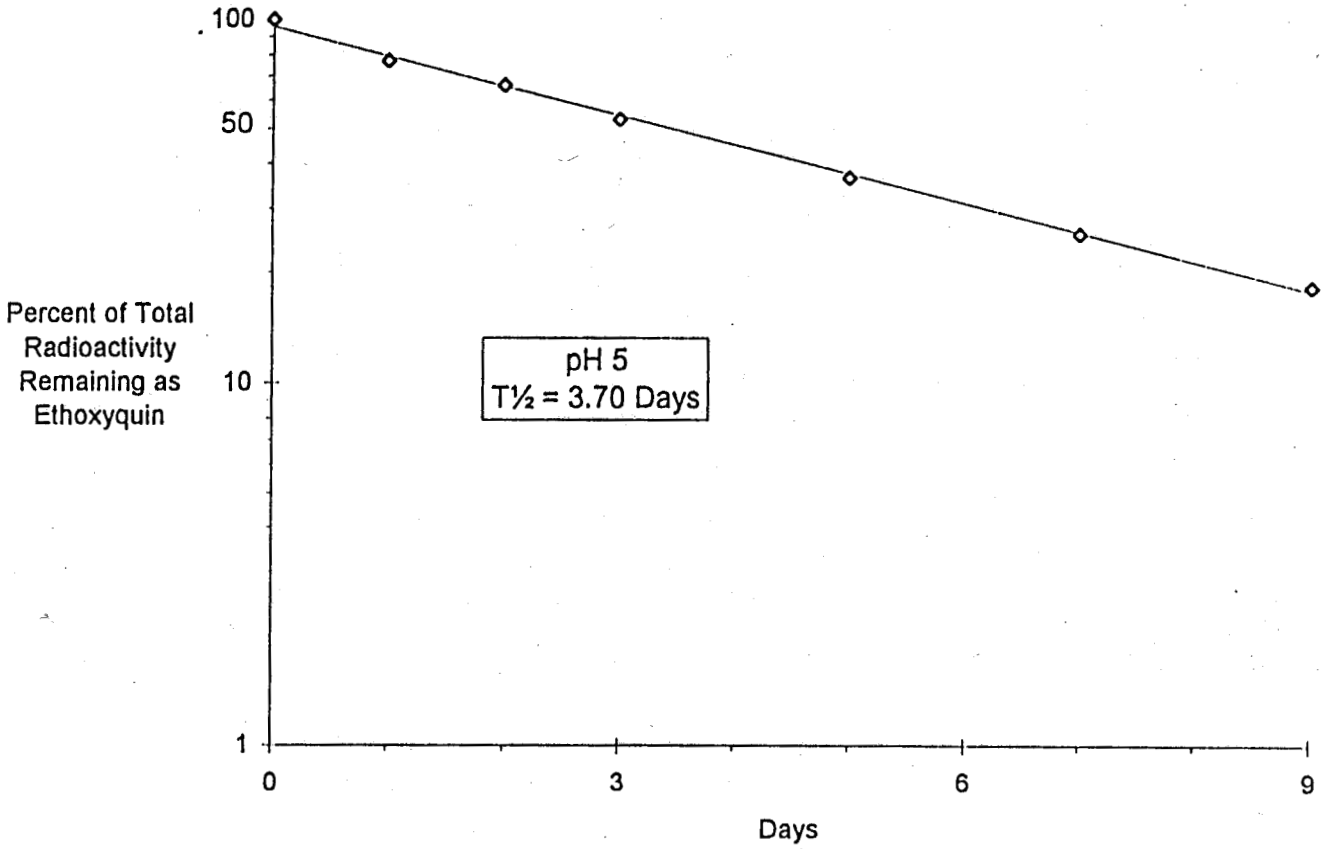


Figure 32

Schematic Illustration of Half-life Regression Analysis for pH 7

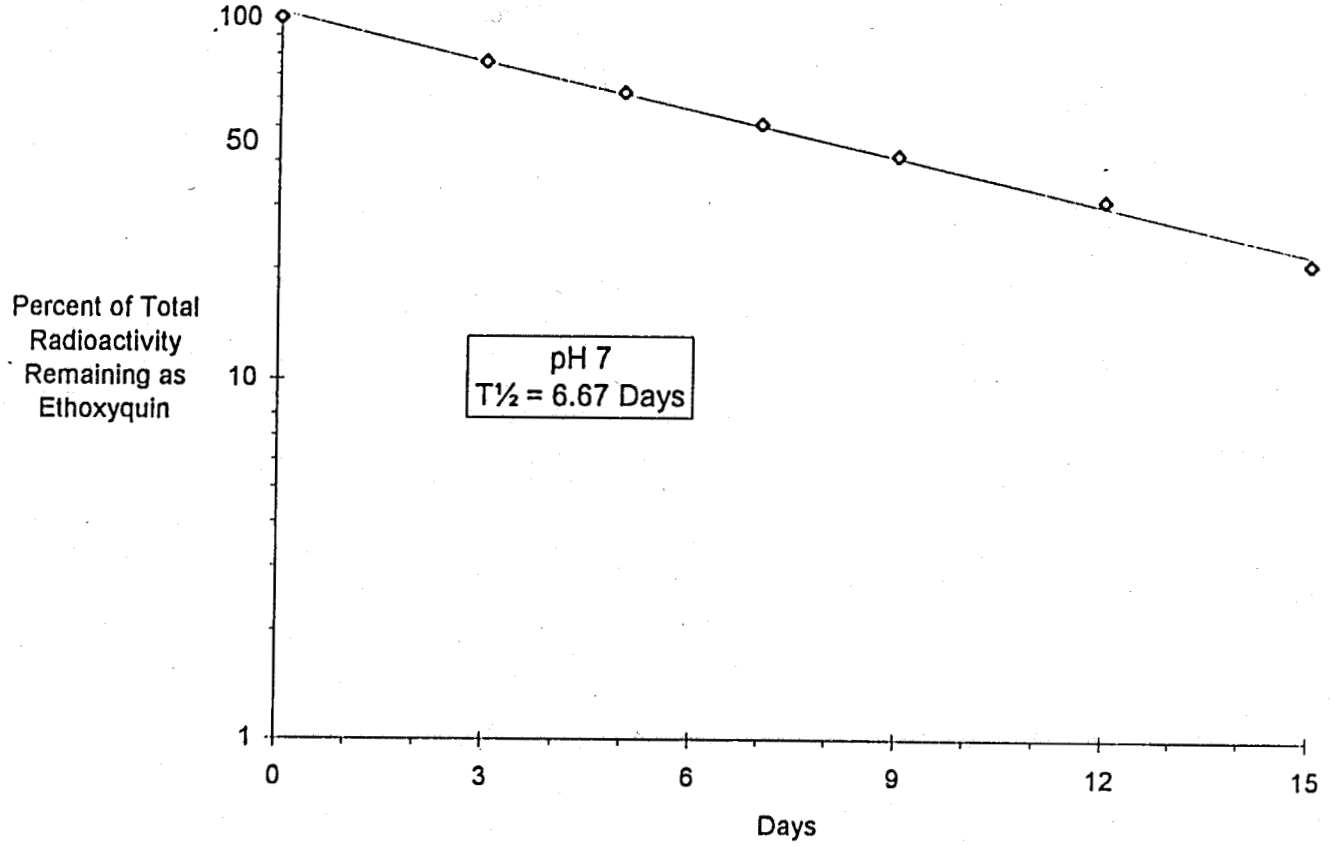
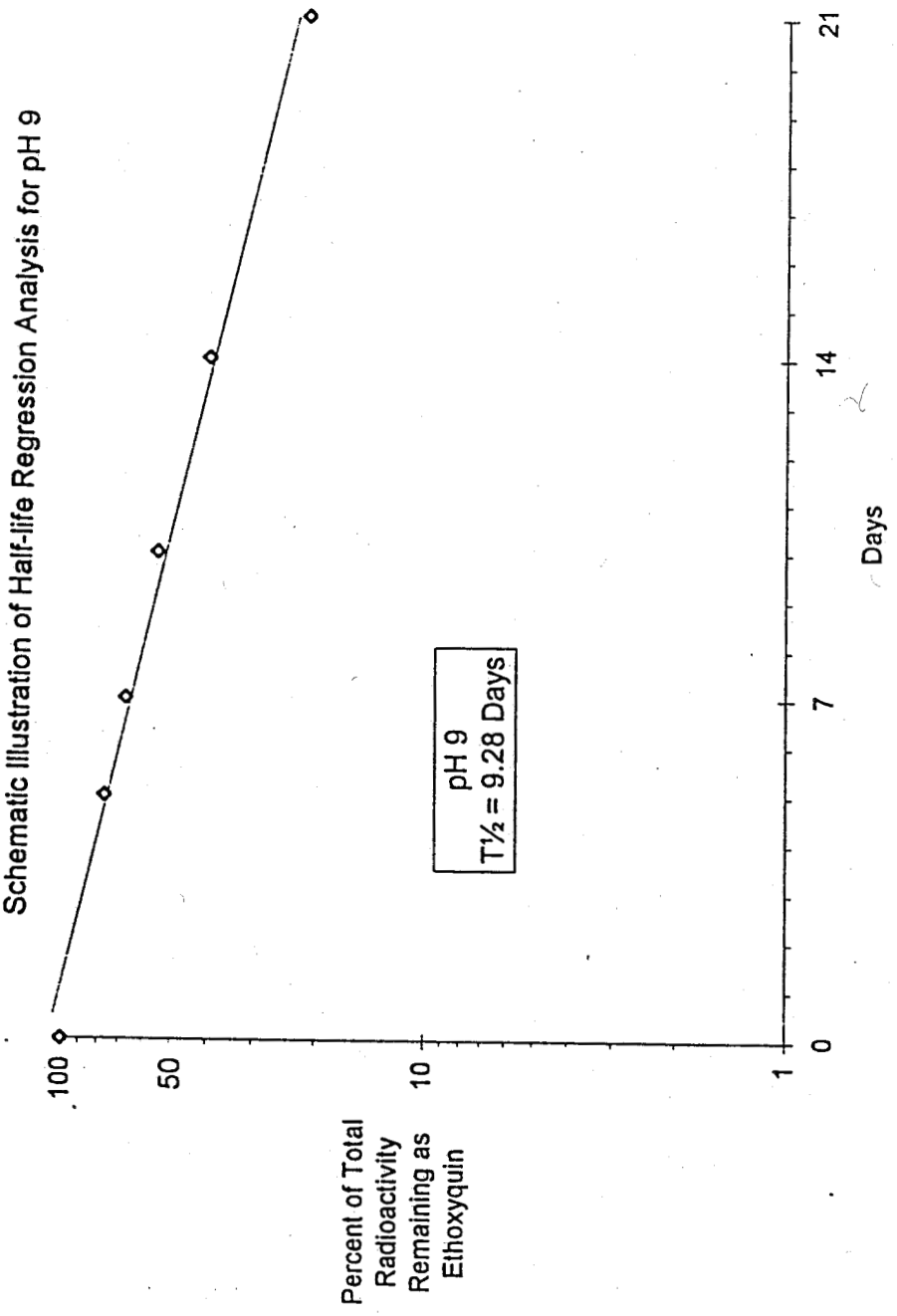


Figure 33

Schematic Illustration of Half-life Regression Analysis for pH 9



APPENDIX C

XenoBiotic Laboratories, Inc.
XBL95011
Summary of Average Daily Temperatures
Incubator #8

	Temp. °C[1]
Average	25.30
Std. Dev.	0.33

Date	Temp. °C[1]	Date	Temp. °C[1]	Date	Temp. °C[1]
02/06/95	25.36	03/08/95	25.91	04/07/95	24.71
02/07/95	25.39	03/09/95	26.14	04/08/95	24.83
02/08/95	25.25	03/10/95	26.11	04/09/95	24.85
02/09/95	25.21	03/11/95	25.83	04/10/95	25.04
02/10/95	25.33	03/12/95	25.79	04/11/95	25.21
02/11/95	25.26	03/13/95	25.76	04/12/95	25.18
02/12/95	25.25	03/14/95	25.81	04/13/95	25.03
02/13/95	25.31	03/15/95	26.04	04/14/95	25.00
02/14/95	25.39	03/16/95	26.11	04/15/95	25.04
02/15/95	25.30	03/17/95	26.08	04/16/95	25.05
02/16/95	25.33	03/18/95	25.15	04/17/95	24.97
02/17/95	25.22	03/19/95	25.13	04/18/95	24.96
02/18/95	25.35	03/20/95	25.19	04/19/95	24.92
02/19/95	25.32	03/21/95	25.13	04/20/95	25.15
02/20/95	25.32	03/22/95	25.11	04/21/95	25.40
02/21/95	25.44	03/23/95	25.12	04/22/95	25.49
02/22/95	25.32	03/24/95	25.07	04/23/95	25.46
02/23/95	25.39	03/25/95	25.07	04/24/95	25.47
02/24/95	25.35	03/26/95	25.06	04/25/95	25.40
02/25/95	25.43	03/27/95	24.91	04/26/95	25.40
02/26/95	25.47	03/28/95	24.88		
02/27/95	25.37	03/29/95	24.87		
02/28/95	25.33	03/30/95	24.94		
03/01/95	25.37	03/31/95	25.01		
03/02/95	25.49	04/01/95	25.13		
03/03/95	25.51	04/02/95	25.09		
03/04/95	25.38	04/03/95	NA		
03/05/95	25.48	04/04/95	24.84		
03/06/95	25.47	04/05/95	24.85		
03/07/95	25.55	04/06/95	24.84		

NA = Not Available

[1] Automatic data were collected at hourly intervals. The hourly data from 9:00 am on the previous day to 9:00 am on the dates listed were automatically averaged.

APPENDIX K

VERIFICATION OF TEST SYSTEM STERILITY

APPENDIX K

REPORT NUMBER
F95116-509
ACCOUNT NUMBER
94000

A & L GREAT LAKES LABORATORIES, INC.
3505 Conestoga Drive • Fort Wayne, Indiana 46808-4413 • Phone 219-483-4759

SEND TO:

XENOBIOTIC LABS INC
JOANNE L. REYNOLDS
107 MORGAN LANE
PLAINSBORO NJ 08536

XBL 95011

Lab Number: 095412
Sample ID: 509A PH5

REPORT OF ANALYSIS

Date Sampled : 03/30/95
Date Received: 04/26/95
Date Reported: 04/28/95 Page: 1

Parameter	Result	Units	Method Detection Limit	Analyst	Analysis Date	Method Reference
Heterotrophic Plate Count	0	col/1ml		JAS	04/26/95	SM(16th)-907A

REPORT NUMBER
F95116-509
ACCOUNT NUMBER
94000

A & L GREAT LAKES LABORATORIES, INC.

3505 Conestoga Drive • Fort Wayne, Indiana 46808-4413 • Phone 219-483-4759

SEND TO:

XENOBIOTIC LABS INC
JOANNE L. REYNOLDS
107 MORGAN LANE
PLAINSBORO NJ 08536

XBL 95011

REPORT OF ANALYSIS

Lab Number: 095413
Sample ID: 509B PH5

Date Sampled : 03/30/95
Date Received: 04/26/95
Date Reported: 04/28/95 Page: 2

Parameter	Result	Units	Method Detection Limit	Analyst	Analysis Date	Method Reference
Heterotrophic Plate Count	0	col/1ml		JAS	04/26/95	SM(16th)-907A

REPORT NUMBER
F95116-509
ACCOUNT NUMBER
94000

A & L GREAT LAKES LABORATORIES, INC.

3505 Conestoga Drive • Fort Wayne, Indiana 46808-4413 • Phone 219-483-4759

SEND TO:

XENOBIOTIC LABS INC
JOANNE L. REYNOLDS
107 MORGAN LANE
PLAINSBORO NJ 08536

XBL 95011

REPORT OF ANALYSIS

Lab Number: 095414
Sample ID: 709A PH7

Date Sampled : 04/18/95
Date Received: 04/26/95
Date Reported: 04/28/95

Parameter	Result	Units	Method Detection Limit	Analyst	Analysis Date	Method Reference
Heterotrophic Plate Count	0	col/1ml		JAS	04/26/95	SM(16th)-907A

REPORT NUMBER
F95116-509
ACCOUNT NUMBER
94000

A & L GREAT LAKES LABORATORIES, INC.

3505 Conestoga Drive • Fort Wayne, Indiana 46808-4413 • Phone 219-483-4759

SEND TO:

XENOBIOTIC LABS INC
JOANNE L. REYNOLDS
107 MORGAN LANE
PLAINSBORO NJ 08536

XBL 95011

REPORT OF ANALYSIS

Lab Number: 095415
Sample ID: 709B PH7

Date Sampled : 04/18/95
Date Received: 04/26/95
Date Reported: 04/28/95

Parameter	Result	Units	Method Detection Limit	Analyst	Analysis Date	Method Reference
Heterotrophic Plate Count	0	col/1ml		JAS	04/26/95	SM(16th)-907A

REPORT NUMBER
F95116-509
ACCOUNT NUMBER
94000

A & L GREAT LAKES LABORATORIES, INC.

3505 Conestoga Drive • Fort Wayne, Indiana 46808-4413 • Phone 219-483-4759

SEND TO:

XENOBIOTIC LABS INC
JOANNE L. REYNOLDS
107 MORGAN LANE
PLAINSBORO NJ 08536

XBL 95011

REPORT OF ANALYSIS

Lab Number: 095416
Sample ID: 90SA PH9

Date Sampled : 04/11/95
Date Received: 04/26/95
Date Reported: 04/28/95

Parameter	Result	Units	Method Detection Limit	Analyst	Analysis Date	Method Reference
Heterotrophic Plate Count	0	col/1ml		JAS	04/26/95	SM(16th)-907A

REPORT NUMBER
F95116-509
ACCOUNT NUMBER
94000

A & L GREAT LAKES LABORATORIES, INC.

3505 Conestoga Drive • Fort Wayne, Indiana 46808-4413 • Phone 219-483-4759

SEND TO:

XENOBIOTIC LABS INC
JOANNE L. REYNOLDS
107 MORGAN LANE
PLAINSBORO NJ 08536

XBL 95011

Lab Number: 095417
Sample ID: 908B PH9

REPORT OF ANALYSIS

Date Sampled : 04/11/95
Date Received: 04/26/95
Date Reported: 04/28/95

Parameter	Result	Units	Method Detection Limit	Analyst	Analysis Date	Method Reference
Heterotrophic Plate Count	0	col/1ml		JAS	04/26/95	SM(16th)-907A